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APPLICATION NUMBER: *60/497,917*
FILING DATE: *August 27, 2003*

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH
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PATENT APPLICATION SERIAL NO. _____

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FEE RECORD SHEET

08/28/2003 EAREGAY1 00000048 60497917

01 FC:2005 80.00 OP

PTO-1556
(5/87)

*U.S. Government Printing Office: 2002 — 489-267/69033

PTO/SB/16 (05-03)
Approved for use through 4/30/2003. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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16424 U.S. PTO
60/497917

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

Express Mail Label No.

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
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☐ *Additional Inventors are being named on the _____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

DIAGNOSTIC DEVICE

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 26 ☐ CD(s), Number

☒ Drawing(s) *Number of Sheets* 12 ☐ Other (specify)

☒ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27.

☒ A check or money order is enclosed to cover the filing fees

FILING FEE AMOUNT ($) 80.00

☒ The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 022095

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____.

Respectfully submitted,

SIGNATURE _____ Date August 26, 2003

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TELEPHONE 416-364-7311 Docket Number: 13340-2

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

08/27/03 16998 U.S. PTO

PTO/SB/17 (05-03)
Approved for use through 04/30/2003. OMB 0651-0032
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FEE TRANSMITTAL for FY 2003

Effective 01/01/2003. Patent fees are subject to annual revision.

☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80.00

Complete if Known	
Application Number	n/a
Filing Date	n/a
First Named Inventor	Wei Hu
Examiner Name	n/a
Art Unit	n/a
Attorney Docket No.	13340-2

METHOD OF PAYMENT *(check all that apply)*

☑ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☐ Deposit Account:

Deposit Account Number: 022095

Deposit Account Name: Bereskin & Parr

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	750	2001	375	Utility filing fee	
1002	330	2002	165	Design filing fee	
1003	520	2003	260	Plant filing fee	
1004	750	2004	375	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80.00

SUBTOTAL (1) ($) 80.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

		Extra Claims		Fee from below	Fee Paid
Total Claims	- 20 ** =		X		= 0.00
Independent Claims	- 3 ** =		X		= 0
Multiple Dependent					=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	84	2201	42	Independent claims in excess of 3
1203	280	2203	140	Multiple dependent claim, if not paid
1204	84	2204	42	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	410	2252	205	Extension for reply within second month	
1253	930	2253	465	Extension for reply within third month	
1254	1,450	2254	725	Extension for reply within fourth month	
1255	1,970	2255	985	Extension for reply within fifth month	
1401	320	2401	160	Notice of Appeal	
1402	320	2402	160	Filing a brief in support of an appeal	
1403	280	2403	140	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,300	2453	650	Petition to revive - unintentional	
1501	1,300	2501	650	Utility issue fee (or reissue)	
1502	470	2502	235	Design issue fee	
1503	630	2503	315	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	750	2809	375	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	750	2810	375	For each additional invention to be examined (37 CFR 1.129(b))	
1801	750	2801	375	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($) 0

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Patent Application Data Sh_ _t

Application Information

Application Type::	Provisional
Subject Matter::	Utility
Computer Readable Form (CRF)?::	Yes
Title::	DIAGNOSTIC DEVICE
Attorney Docket Number::	13340-2
Total Drawing Sheets::	12
Small Entity?::	Yes

Applicant Information

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Initial DD/MM/YY

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BERESKIN & PARR

B&P File No. 13340-2

UNITED STATES

Title: DIAGNOSTIC DEVICE
Inventor: Wei Hu

DIAGNOSTIC DEVICE

This application is a continuation-in-part of application Serial Number 10/279,566, filed Octob r 24, 2002.

FIELD OF THE INVENTION

This invention relates to diagnostic devices, and in particular, to diagnostic devices that allow for the detection of analytes in liquid samples, such as body samples and environmental samples.

BACKGROUND OF THE INVENTION

A wide variety of devices for detecting the presence of analytes in a liquid sample, such as body samples and environmental samples, through the use of immunochemistry have been recently developed. Typically, these devices perform the *in vitro* diagnostic test on the surface of a dry porous carrier, such as a sheet or strip of nitrocellulose membrane, contained in a housing having a sample deposition site and a detection site for viewing the assay result(s). A sample is applied as a liquid drop to one end of the carrier, and flows by capillary action downstream to the other end passing a reagent immobilized in between. As the sample advances along the strip, additional mobile reagents disposed on the carrier bind to the analyte and become entrained in the sample flow. The assay is read by observing the presence of the analyte-binding reagent at the detection site.

More recently, it has been observed that devices which encourage confocal sample flow through the mobile reagents on the carrier are advantageous, because such devices concentrate the reagents and analytes and retard the migration of particulates, such as red blood cells, thereby enhancing the reliability of the test results. The presence of red blood cells in the detection channel interferes with the proper visualization of the test results because of the intense hue of the cells.

Several devices have been developed having liquid sample deposition structures which directly or indirectly promote the confocal flow of liquid sample, or the converging of sample flow, towards the detection channel of

the carrier. For instance, a device including a U-shaped sample deposition means from which sample can be deposited across a carrier for capillary flow into a narrowed detection channel produces a generally confocal sample flow. However, given that diagnostic devices of this type are intended to be disposable after a single use, and, thus, inexpensive, the engineering cost necessary to manufacture such a U-shaped sample deposition means can be prohibitively high.

Accordingly, there remains a need for a diagnostic device which is inexpensive to manufacture and easy to use, and which encourages the efficient separation and/or filtration of particulates so as to provide reliable test results.

SUMMARY OF THE INVENTION

The present invention is directed to a diagnostic device for testing a liquid sample having a carrier for receiving at least a portion of the sample and a sample deposition apparatus. The sample deposition apparatus has an injection channel that is in fluid communication with a deposition channel. The depth of the deposition channel is determined by a deposition channel defining surface, which surface is spaced apart from the carrier such that at least a portion of the sample disperses in the deposition channel by capillary action to form a sample band.

In a preferred embodiment, the space between the deposition channel defining surface and the carrier is less than 0.5 mm, and more preferably is 0.1 mm. The sample deposition channel may be generally rectilinear.

The present invention is also directed to a diagnostic device for testing a liquid sample having a carrier for receiving at least a portion of the sample and a sample delivery apparatus. The sample delivery apparatus has a conduit that is in fluid communication with the carrier, and further has an inlet and an outlet. The conduit has a depth and a width, wherein the ratio of the width to the depth is less than 1.0.

In a preferred embodiment, the ratio of conduit width to depth is less than 0.5. The ratio of the conduit width to depth at the outlet may be less than the ratio at the inlet. The depth of the conduit may increase along the length thereof. The volumetric capacity of the conduit may be designed to provide sufficient sample for testing.

BRIEF DESCRIPTION OF THE DRAWINGS

For a better understanding of the present invention and to show clearly how it may be carried into effect, reference will now be made, by way of example, to the accompanying drawings which show a preferred embodiment of the present invention in which:

Figure 1 is an exploded perspective view of the components of a device in accordance with a preferred embodiment of the present invention;

Figure 2 illustrates a perspective view of a pad arrangement of the device of Figure 1;

Figure 3 illustrates a top plan view of a pad arrangement of the device of the device of Figure 1;

Figure 4 illustrates a side elevation view of a pad arrangement of the device of Figure 1;

Figure 5 illustrates a perspective view of a pad arrangement including a wicking pad;

Figure 6 is a top perspective view of the outer surface of the top member of the device of the present invention, showing the sample delivery means;

Figure 7 is a sectional side view of the conduit of the device of Figure 1;

Figure 8 is a sectional side view of the device of Figure 1 with a first alternate conduit;

Figure 9 is a sectional side view of the device of Figure 1 with a second alternate conduit;

Figure 10 is a sectional side view of the device of Figure 1 with a third alternate conduit;

Figure 11 is a sectional front view of the injection channel apparatus of the subject device;

Figure 12 is a sectional side view of the injection channel apparatus and sample deposition apparatus of the subject device;

Figure 13 is a top perspective view of the inner surface of the top member of the subject device;

Figure 14 is a sectional side view of the sample deposition apparatus of the subject device;

Figure 15 is a top perspective view of the base member of the subject device;

Figure 16 illustrates the use of subject device to receive a liquid sample from a patient;

Figure 17 illustrates the labeled antibodies migrating in the sample flow using a device made in accordance with an embodiment of the present invention;

Figure 18 illustrates the results obtained using a device made in accordance with an embodiment of the present invention with a normal human serum sample;

Figure 19 illustrates the results obtained using a device made in accordance with an embodiment of the present invention with a normal human serum sample spiked with 6.25ng/ml of rCKMB;

Figure 20 illustrates the results obtained using a device made in accordance with an embodiment of the present invention with a normal human serum sample spiked with 32.35 ng/ml of rCKMB; and

Figure 21 illustrates the results obtained from the experiment described in Example 3.

DETAILED DESCRIPTION OF THE INVENTION

The device of the present invention may be employed to analyze a variety of liquid samples, especially biological samples. These include environmental samples, such as wastewater, and medical samples that include blood, its components, urine, cerebrospinal fluid, etc. Further, the device is especially useful for detecting the presence of analytes in a liquid sample containing at least a portion of whole blood, serum and /or plasma, such analytes including, for instance, myoglobin, troponins including TnI, TnT, and TnC, myosin light chain, fatty acid binding protein, actin, CK-MB, CA-III, BNP, and the like, as

well as markers of viral, bacterial, fungal and tumour burden, such as PSA, her-1 and her-2. In another embodiment, the device is utilized to detect urine-borne analytes, including hCG, LH, GnRH, drugs of use and abuse, markers of metabolism such as glucose , and the like. The reagents required to conduct assays for these analytes are all available commercially.

Reference is made to Figures 1, 2, 3 and 7 which illustrate a diagnostic device 10 made in accordance with a preferred embodiment of the invention. The device 10 comprises a housing 12 formed of a base member 14 and a top member 16 that are mateable by friction fit using connectors 18 provided on the top member 16 and corresponding recesses 20 provided on the base member 14. Any other means known in the art may be employed to permanently or removably fix the members 14 and 16 together. The housing 12 is of a size convenient for holding the device 10 in one hand during the operation of the test. In the embodiment depicted in Figures 1 and , the actual device is about one-third the size shown

The housing 12 accommodates a porous carrier 22 which is held between the members 14 and 16. The porous carrier 22 is maintained in the housing 12 in sufficient alignment to allow for the diagnostic test to be performed, using a plurality of carrier stabilizers 17 formed on bottom member 14. At least one alignment means 19 corresponding to a carrier stabilizer 17 is provided on the base member 14.

The carrier 22 may be any suitable carrier material known in the art having a surface for conducting the flow of a liquid sample. For instance, the carrier 22 may be formed of glass fibers, nitrocellulose membranes, or any suitable polymeric material on which liquid sample can flow desirably by capillary action. Preferably, the carrier 22 is comprised of an array of pads coupled in flow communication. The array comprises a sample pad 24 for receiving at least a portion of the sample and a detection channel 26 having a transverse width *X* that is reduced relative to the width *Y* of the sample pad 24. The detection channel 26 includes a bridging pad 28, a detection channel pad 30 with a capture zone 32. The bridging pad 28 has a sample conducting

surface 29 (Figure 2). The detection channel 26 may be designed to have a detection channel pad 30 that provides sufficient capillary draw of the liquid sample. A wicking pad 34 may also be provided in flow communication with the detection channel 26 to draw sufficient sample across the capture zone 32 and maintain sample flow along the detection channel 26. Use of the wicking pad 34 may have the effect of reducing the length of the detection channel 26, and the overall device, by substituting for an elongated detection pad.

As shown in Figures 1 and 2, a layer of barrier material 35, that can enhance flow of liquid thereunder. The barrier material 35 should be transparent or translucent, and a suitable material is hydrophilic adhesive tape. The barrier material 35 is applied along substantially the entire length of the detection channel 26. This ensures that the bridging pad 28 and the detection channel pad 30 remain in flow communication. In addition, and importantly, it has been found that the barrier material 35 has the effect of enhancing the flow of sample thereunder, and thus has the advantage of effectively drawing sample into the detection channel 26 from the sample pad 24. The barrier layer 35 also prevents accidental smearing or damage or other exposure to the carrier 22 exposed at the observation window 34.

Each pad 24, 28 and 30 within the array constituting the porous carrier 22 may be formed of the same carrier material, however, different carrier materials and carrier compositions may be used. For instance, the pads 24, 28 and 30 can be formed of glass fibers, of nitrocellulose, or of any suitable polymeric material on which liquid sample can flow desirably by capillary action. The carrier 22 can be made of material suitable for filtering sample as it migrates, or for allowing sample particulates to separate chromatographically as sample migrates therealong. This is of particular benefit when the applied sample is blood. In this case, the structure of the carrier material preferably functions to separate the blood components chromatographically, causing the formation of a plasma front advancing ahead of red cells and other particulate material.

In the embodiments illustrated in Figures 2, 3, 4 and 5, the pads 24, 28 and 30 are each formed of nitrocellulose having an average pore size in the 1-10 micron range, e.g., of about 3 microns or about 5 microns. The pads are cut from a larger sheet of nitrocellulose having a backing material 37 that is water-impermeable, to prevent sample leakage, and provides some rigidity to the otherwise supple nitrocellulose material. A suitable such material is polyester film. The pads illustrated in Figures 2, 3, 4 and 5 thus have a backing material, and present only one surface, or face, on which the assay can be conducted. Additionally, the sample conducting faces of the sample pad 24 and the detection pad 30 are bridged between and in contact with the sample conducting face of the bridging pad 28. The pads 24, 28 and 30 overlap at their edges to establish sample flow communication along the length of the carrier 22. By placing the bridging pad above the sample pad 24, there is provided a capillary "lift" that assists with filtration of the sample and, in the case of a blood sample, further accentuates the chromatographic separation of the sample into a leading plasma front and trailing red blood cells.

Alternatively, the detection channel 26 may comprise a single detection pad having dimensions comparable to the combined bridging pad 28 and detection channel pad 30, and the wicking pad 34 when required. To couple a single detection pad to the sample pad, any backing material on the detection pad can be removed at least at the interface thereof with the sample pad, to foster sample flow from the sample pad into the detection pad defining the detection zone.

The carrier is provided with one or more reagents. The reagents may be any mobile or immobile analyte-binding reagents suitable for detecting the analytes of interest and/or for eliminating interfering factors. As shown in Figure 3, one or more different mobile detector reagents can be deposited as individual bands spanning the width of the sample pad 24. In the alternative shown in Figure 3, these detector reagents can be deposited as reagent blots. Moreover, the mobile reagents can be deposited at any site on the carrier that is upstream of the site at which the immobilized reagents are deposited, for

instance at an upstream site on the detector pad, on the bridging pad or, desirably, on the sample pad at a position downstream of the sample deposition site. At least one of the reagents is preferably coupled to a label that can be detected either visually or with suitable instrumentation. Any of a variety of labels known to a person skilled in the art may be utilized in the device of this invention. Preferred reagents are antibodies or any other reagent that binds the analyte, labeled with metal and enzyme labels, such as gold sol, fluorophore or lumiphore labels. Metal labels are especially preferred due to their sensitivity. A gold label can be enhanced to become more readily visible by the use of a soluble silver salt and a reducing agent in accordance with known procedures. Alternatively, an enzyme label, such as horseradish peroxidase, may be detected by the addition of hydrogen peroxide and a dye, such as ortho phenylenediamine, in accordance with standard procedures.

It will be appreciated that the immobilized capture reagent can be deposited in the shape of any desired indicia, and is illustrated in Figure 3 as a straight line. To control for false negative results, the detection pad 30 can further comprise a control line 33, bearing an immobilized reagent that is non-specific for analyte, but indicates that sample has migrated successfully past the analyte capture zone. A binding partner specific for a mobile detector reagent deposited in the sample pad can serve this purpose.

The performance of a typical analyte detection test involves depositing at least a portion of a liquid sample on sample pad 24 having the one or more mobile reagents provided thereon. These reagents bind to analytes present in the liquid sample to form analyte-reagent complexes and becomes entrained in the sample flow for movement with the analytes. The complexes advance with the liquid sample flow by capillary action towards bridging pad 28 and migrate into the detection channel 26. In the detection channel 26, the complexes encounter one or more analyte-binding capture reagents immobilized on capture zone 32. The accumulation of each label from the analyte-reagent complexes at a respective immobilized capture reagent in the capture zone 32 reports a test result, confirming that either the targeted

analyte or one of the mobile reagents is present in the liquid sample under investigation. An observation window 34 may be provided in top member 16 corresponding to capture zone 36 to allow the results of the test to be viewed either visually or with suitable instrumentation. The detection of the presence of at least one analyte enables a physician to characterize the cardiac event, for example, as stable or unstable angina or as a myocardial infarction.

The reliability of a diagnostic test of this type depends on depositing a sufficient amount of liquid sample on the carrier, separating any particulates, such as red blood cells, from the liquid sample so as to permit the plasma containing the labeled analyte-reagent complex to advance to the detection channel, and blocking interfering factors. Efficient separation of red blood cells from the liquid sample is particularly important because red blood cells are strongly coloured and, thus, tend to interfere with the viewing and interpretation of the test results.

In embodiments the device of the present invention provides improved structures for the delivery and deposition of liquid samples on carrier 22, and particulate filtration and separation structures that more efficiently remove particulates from the liquid sample, such structures are now being described in greater detail.

Figures 6 and 13 illustrate the top member 16 of the device 10 having an outer surface 38 and an inner surface 40. The outer surface 38 is provided with an observation window 34 and a sample delivery means 42. The sample delivery means 42 comprises a conduit 44 with a generally V-shaped (in plan view) channel having a bed 46, a pair of side walls 48, and a top that is open to the ambient environment. The bed 46 of conduit 44 is designed to provide increasing depth along the length thereof. As best shown in Figure 11, bed 46 has a depth that increases at an angled slope 49 along the length of the conduit 44. Accordingly, when the device is placed on a horizontal surface, there is a gravitational tendency for sample to advance along the conduit, aided by capillary action.

Returning to Figure 6, the conduit 44 further comprises an inlet 50 and an outlet 52. The inlet 50 extends outwardly from outer surface 38 and comprises flanges 54 and 56. The inlet 50 receives the liquid sample that is to be tested. At least a portion of the sample received at the inlet 50 advances along the conduit 44 to the outlet 52. Flanges 54 and 56 are notched to facilitate liquid sample entering into conduit 44.

Additionally, the conduit 44 has a generally V or an open hairpin shape such that when the device 10 is held by the user in an angled orientation, as may be desirable when a liquid sample is received in the conduit for testing, there is a further tendency for at least a portion of the sample to move by gravity along the conduit. Any other suitable conduit design may be adopted to this end, including, for example, a linear or curved conduit.

To encourage this type of flow, it will be appreciated that top member 16 is suitably formed of any material that is wettable and can be machined or otherwise shaped to introduce the features of the present sample delivery system. Suitable such materials are in common use in the diagnostics industry and include hydrophilic plastics material, such as acrylic, including methacrylates and polymethacrylates. Conversely, the base member 14 of the housing 12 is desirably formed of machinable, hydrophobic plastics material to repel diffusion of sample onto the base member from the carrier 22. Suitable such materials include polystyrene.

Figure 7 shows one profile for the conduit 44, comprising the bed 46 and the side walls 48 having a deep rectangular cross-section. Conduit width B should preferably be less than the conduit depth A so that the ratio of B to A is equal to or less than 1.0. Most preferably the ratio of B/A is equal to or less than 0.5. The conduit 44 desirably has a volume capable of holding sufficient liquid sample for the performance of any given diagnostic test. It will be appreciated that this volume can be adjusted by increasing one or more of the width, depth or length of the conduit. It should be appreciated that the conduit 44 can have various cross-sections. Figures 8, 9 and 10 illustrate a first, second, and third alternate conduit. Referring to Figure 8, a first alternate

conduit 144 has a trough-like cross-section with a top width C and a bottom width D, wherein the top width C is greater than the bottom width D. The first alternate conduit 144 has outwardly bowed or slanted side portions 49. Figure 9 shows a second alternate conduit 244 comprising a depth E, top width F and a bottom width G. The top width F is greater than the bottom width G. The second alternate conduit 244 has a curved or semi-circular base 51 which reduces the amount of sample that may stagnate in the conduit. Figure 10 illustrates a third alternate conduit 344 having a trough-like cross-section and a top width H and bottom width J. Bottom width J is less than top width H, and bottom width J is provided with a V-shaped groove along the base 53 of the conduit. A conduit shaped in accordance with the third alternate conduit 344 is also efficient at minimizing the amount of stagnation that occurs in the conduit.

Additionally, it will be appreciated that the top member 16 may include a protective layer of material, such as a fixed or removable plastic shield or adhesive tape (not shown), to cover the exposed conduit 44 to prevent contamination and tampering of the liquid sample. The protective layer is preferably translucent to enable the viewing of the sampling in the conduit by the user.

The outlet 52 of the conduit 44 is in fluid communication with an injection channel means 58. As best shown in Figures 11 and 12, the injection channel means 58 of the device 10 extends from the outer surface 38 through to the inner surface 40 of the top member 16. The injection channel means 58 comprises a reservoir 60, a capillary injection channel 62 and an injection aperture 66. The reservoir 60 is conical or funnel shaped having a circumference that decreases toward the injection channel. Flow of at least a portion of the sample through the reservoir 60 to injection channel 62 is achieved by exploiting a combination of surface tension minimization, capillary action, and gravity.

The reservoir 60 may be designed to have a volumetric capacity that is capable of controlling the flow of the liquid sample into the injection channel

62 while, together with conduit 44, containing sufficient liquid sample for performing the diagnostic test. In general, modulation or control of the flow of the liquid sample may be achieved by different means. For instance, the flow may be controlled by constricting the cross-section of the injection channel through which the liquid sample flows.

As shown in Figures 13 and 14, the injection aperture 66 of injection channel 62 is in fluid communication with a sample deposition means 68 provided in the inner surface 40 of top member 16. The injection aperture 66 is shown centered in the sample deposition means 68. However, it will be appreciated that injection channel means 58 may be designed to provide a sample flow to the sample deposition means 68 at any position. By the design illustrated, the liquid sample received at inlet 50 is drawn by surface tension minimization, capillary action and gravity into the conduit 44, advances therealong by capillary action and gravity through the outlet 52 into reservoir 60. The reservoir 60 then fills and empties by surface tension minimization, gravity and capillary action into the injection channel 62, and then passes through the injection aperture 66 onto the sample deposition means 68, which then fills by capillary action between the sample deposition means 68 and sample pad 24.

Figures 13 and 14 further illustrate that the sample deposition means 68 formed on the inner surface 40 of top member 16 has a deposition channel 70 and a deposition channel defining surface 72 which extends over at least a portion of sample pad 24 of carrier 22. The deposition channel 70 is closed at both ends 74 and has an inner wall 76, an outer wall 78 and a sill 80. The sample pad 24 comprises an operative surface 82 which is positioned to contact sill 80 of sample deposition means 68 so that sample pad 24 is registered directly under injection aperture 66, to receive sample therefrom. The shape of deposition channel 70 corresponds to the shape of the deposition channel defining surface 72. The deposition channel defining surface 72 has a beveled, trailing edge 84.

Abutment of the sill 80 and the operative surface 82 of sample pad 24 defines the depth of the deposition channel 70, such that deposition channel defining

surface 72 is spaced apart from operative surface 82 allowing at least a portion of the liquid sample flow from injection aperture 66 to disperse in deposition channel 70. Preferably, the deposition channel 70 between the deposition channel defining surface 72 and the sample pad 24 of carrier 22 has a depth equal to or less than 1.0 mm. More preferably, the depth between the deposition channel defining surface 72 and sample pad 24 is equal to or less than 0.5 mm, and most preferably the depth is 0.1 mm. The flow of liquid sample is confined to the deposition channel 70 due to the effect of capillary forces, such as a capillary trap, which delay the liquid sample from advancing downstream towards the detection channel 26 beyond the trailing edge 84 of the deposition channel defining surface 72, at least until the deposition channel is substantially filled with sample.

As shown in Figures 13 and 14, the width *M* to length *N* of the deposition channel defining surface is preferably 10. More preferred, the ratio M/N is equal to or less than 7. As such, the walls 76 and 78 and leading edge 84 end at an air junction edge 86, which also delays the liquid sample from flowing downstream beyond the walls and deposition channel defining surface 72, thereby allowing the liquid sample to continue to disperse by capillary action in the deposition channel 70. The liquid sample continues to disperse in and fill the deposition channel 70 to form a generally linear sample band. The sample band corresponds to the shape of the deposition channel defining surface 72 of the deposition channel 70.

The present deposition channel avoids the need to machine a capillary channel that is integral within the top member itself, so that sample is distributed uniformly within the channel before being permitted passage onto the carrier. Rather, it is now realized that it is simpler to form a capillary channel, to promote lateral flow of the sample, between the channel defining surface 72 and the pad 24. The capillary action causing lateral distribution of the sample is rapid enough that the sample starts to be absorbed into the pad 24 across the entire width of the pad 24 almost simultaneously, i.e. the portion of the sample first contacting the pad 24 adjacent the injection channel 62 does not have any significant lead in absorbing into the pad 24.

In operation, at least a portion of the liquid sample enters the sample deposition means 68 through injection aperture 66 and rapidly disperses by capillary action to the ends 74 of the deposition channel 70. Once the deposition channel 70 has been substantially filled and, accordingly, the capillary draw of the sample pad 24 exceeds that of the deposition channel 70, at least a portion of the sample band advances downstream towards detection channel. The advancing sample band initially advances from deposition channel 70 as a generally linear band which may have a slightly curved liquid frontier with the leading edge at or near the center.

The purpose of the sample deposition means 68 is now apparent. If, in the absence of the capillary trap formed by the deposition channel 70, as defined by the sample pad 24 and the sample deposition channel defining surface 72, an operative surface 82 of sample pad 24 was in direct contact with the injection aperture 66, at least a portion of liquid sample in sample delivery means 42 would flow immediately downstream towards detection channel 26. This uncontrolled liquid sample flow would result in a greater amount of red blood cells advancing to the capture zone, since a narrow width of the flow of liquid sample would not allow the efficient separation of particulates from the plasma front by operative surfaces of the carriers. Additionally, a liquid sample flow having a narrow width may increase the duration of the diagnostic test, and reduce the concentration of analyte crossing the test line.

Figure 15 shows the configuration of the top surface of the base member 14. A flat substantially rectangular area 90 and an elongated area 92 projecting from the top surface of base member hold the porous carrier 22. The height of the sides 94 of the rectangular area 90 is sufficient such that the operative surface 82 of the porous carrier 22 is at the same level as sill 80 of the deposition channel 70. Other configurations are contemplated by the present invention, such as designing the base and top members to hold carrier 22 in the correct orientation such that the recessed rectangular area 90 and elongated area 92 are not necessary. It is only necessary that the base and top members correspond with carrier 22 in between to define the fluid path

from injection channel 62 to detection channel 26. The thickness of the various channels and areas 90 and 92 may be adjusted accordingly.

An important advantage of the present invention is the geometry of the device which, when utilized for a blood sample, provides a generally linear sample band on the sample pad 24. The provision of a generally linear sample band encourages converged flow of the blood sample downstream towards the detection channel 26. A converged flow is beneficial because it forms a flow stream having a red blood cell front and downstream thereof a plasma front. More specifically, the red blood cells in the blood sample are separated chromatographically from the plasma which, in a carrier 22 composed of, for example, a nitrocellulose membrane, flows faster than the red blood cells. Accordingly, as a result of the geometric design of the device, a relatively larger amount of plasma containing the analyte-binding mobile reagents is separated from the whole blood on sample pad 24, providing sufficient time for binding reactions to occur. When the sample band advances downstream towards the detection pad 30 along the sample pad 24, the pattern of sample band flow tends to converge toward detection pad 30. Additionally, by encouraging a converged flow pattern along sample pad 24, the device concentrates the reagents and the analyte and retards red blood cell migration, and thereby enhances red blood cell/plasma separation, and the sensitivity of the diagnostic test for a given volume of sample.

Chromatographic separation of the blood sample may be further achieved using bridging pad 28. As shown in Figures 2, 3, 4 and 5, the operative surface 82 of sample pad 24 and detection pad 30 are "bridged" and in contact with sample conducting surface 29 of bridging pad 28. The pads 24, 28 and 30 may be overlapped at their edges to establish liquid communication along the length of carrier 22. By placing at least a portion of the bridging pad 28 directly above sample pad 24, a capillary "lift" is provided that assists with filtration of the sample, and, in the case of a blood sample, further accentuates the chromatographic separation of the liquid sample into a leading plasma front and trailing red blood cells. Flow communication between each of the pads 24, 28 and 30 of carrier 22 can be maintained by

"pinching" the pads at their overlapping interfaces using structure provided on the base member and/or top member. In addition, flow communication between the pads 28 and 30 of carrier 22 is maintained using a layer of wettable and adhesive barrier material applied substantially along the entire length of desired pad. This ensures that pads 28 and 30 remain in flow communication. It is also possible that an array of pads can be provided that are stacked three or more deep.

Referring to Figure 16, in use, a user holds the present device in one hand with the top of the device facing the user or tilted somewhat toward the user. With the other hand, a drop or more of liquid sample is touched to the inlet 50 and the device is then held in this position until sufficient liquid sample is drawn into the device. For most diagnostic applications, 50 µL of a liquid sample is sufficient to obtain reliable test results. The user is allowed to see the liquid sample advance into and accumulate in reservoir 60. The device is then laid flat on a work surface, and the results of the diagnostic test can then either be viewed or determined instrumentally within about 10-20 minutes by detecting the presence of label at the capture zone 32 corresponding to observation window 38.

It will be recognized by those skilled in the art that the overall dimensions of the sample delivery means 42, injection channel means 58 and sample deposition means 68 may be varied according to the desired use, type of liquid sample and quantity of liquid sample required for the subject test. Furthermore, a device 10 having the features of the sample deposition means 68 can be operated using a carrier 22 that has features different from those herein described. For instance, and for simplicity, a device having the sample deposition means 68 can be operated using a carrier 22 that is a single sheet of material. The single sheet of material can be simply rectangular in shape, and accommodated within a housing adapted to receive it. Alternatively, the single sheet of carrier material can be shaped as herein described, to provide a sample pad that is wider than the integral detection pad. This alternative

carrier design can readily be accommodated by the housing 12 described herein.

In addition, it will be appreciated that the present invention can be adapted to detect more than one analyte in a single test. For this purpose, the carrier 22 of the device will comprise mobile, labeled detector reagents for each analyte deposited on the sample pad 24, and immobilized capture reagents for the resulting analyte complexes, positioned as separate bands or other indicia on the detection channel pad 30 in full view from the observation window 34.

Use of a device of an embodiment of the present invention is now described in detail in the following examples.

EXAMPLES

The following results have been obtained by applying the illustrated device in a model system in which the carrier is comprised of the illustrated pad array, where the sample pad is wider than the detection zone formed by the detection channel pad and the bridging pad (absent a wicking pad and without adhesive tap over the detection channel). In the model system, gold conjugated mouse antibody to CK-MB, a cardiac analyte, is used as the labeled detector reagent, which reveals the pattern of sample flow along the carrier. Immobilized goat antibody to CK-MM was used as capture.

More particularly, gold conjugated mouse anti-CKMB (Spectral Diagnostics, Toronto, Canada) solution (OD_{530}=40) was prepared by mixing one volume of StabilGuard (SurModics, Inc., Eden Prairie, MN, USA) and one volume of mouse anti-CKMB gold conjugate (OD_{530}=80).

As sample pad, a polyester supported cellulose nitrate membrane (PuraBind, 3µm nominal pore size (Whatman International Ltd., Maidstone, Kent, UK) was first blocked by immersion into a blocking solution (StabilCoat (SurModics, CA, USA)/H_20=1/3, v/v). After drying, gold conjugated mouse

anti-CKMB antibody (OD530=40) was deposited as 0.5 µl dots onto the blocked sample pad by manual pipetting and dried at 37°C. For the detection zone, a polyester supported cellulose nitrate membrane (PuraBind, 5µm nominal pore size (Whatman International Ltd., Maidstone, Kent, UK) was first blocked by immersion into a blocking solution (StabilCoat (SurModics, CA, USA)/H20 =1/3, v/v). After drying, capture line was streaked onto the detection pad using an IsoFlow ™ Dispenser (Imagene Technology, Hanover, NH, USA) with an antibody solution containing 2mg/ml goat anti-CKMM (Spectral Diagnotics, Inc., Toronto, Canada), 1% sucrose, and 3% methanol.

The carrier was assembled by putting the sample pad and the detection channel pad with attached bridging pad in the restricted compartments in the base member of the device, respectively, so that the cellulose nitrate layers of the sample pad and the bridging pad are facing each other, and the liquid communication between those layers was secured by pressing the upper member of the device housing into the base member.

EXAMPLE 1

The converging pattern of sample flow toward the detection channel was first confirmed in an experiment in which normal human serum was delivered as a linear band from the deposition channel and then permitted to flow for about ten minutes toward the detection channel. The bridging pad was then removed, thereby stopping sample flow within the sample pad, to reveal the label flow pattern. As illustrated in Figure 17, the labeled detector antibodies entrained within the migrating sample clearly displayed flow converging toward the bridging pad of the detection zone. This same converging sample flow is seen in assays that run to completion without bridging pad interruption. Notwithstanding the reduced width of the detection channel relative to the span of labeled detector antibody deposited on the sample pad, there was very little detectable stagnation of reagent or sample in the "shoulders" or corners of the sample pad. Substantially all of the sample and reagent migrated toward the bridge leading into the detection channel. There is,

accordingly, no need to shape the sample deposition channel for confocal sample flow. A linear sample deposition band is sufficient to drive the desired flow into the detection channel.

EXAMPLE 2

The carrier pad arrangement described in Example 1 was also employed in an embodiment of the present invention for the detection of rCKMB, as analyte. In this assay, 50 µl of normal human serum alone or spiked with rCKMB was tested. The results at 15 minutes are illustrated in Figure 18 (normal human serum), Figure 19 (normal human serum spiked with 6.25ng/ml rCKMB), and Figure 20 (spiked with 32.35 ng/ml rCKMB). Figures 17, 18, 19 and 20 reveal that label deposited on the sample pad flowed almost completely out of the sample pad and into the detection zone, there being very marginal and negligible stagnation of labeled reagents in the "shoulders" or corners of the sample pads. The results also reveal that rCKMB is detected in the spiked samples, the assay being more sensitive to detection of CKMB at the higher concentrations.

EXAMPLE 3

The device also was assessed for its ability to retard the flow of red blood cells, so they do not migrate into and obscure results otherwise visible at the capture line. To this end, 50 µl of fresh heparinized human whole blood was tested. After 15 minutes, the majority of the red blood cells were retained in the sample pad, and the front of the red blood cells was restricted at the center of the bridging pad. After about one hour, the front of the red blood cells was stabilized at just beyond the bridge before reaching the capture line within the read-out window, and remained there afterwards. Hemolsis was not visually detectable. The result at 72 is shown in Figure 21. It will thus be appreciated that the bridging pad and its elevation relative to the sample pad and detection channel pad also contributes to the filtration of sample

particulates, including red blood cells, and that this carrier array is particularly well adapted for the detection of soluble analytes present in blood samples.

While the embodiments of the present invention have been exemplified with reference to a particular diagnostic immunoassay and format, it will be appreciated that any of a variety of lateral flow type assays, including, for example, indirect formats and competitive binding formats, can be conducted to detect a variety of analytes present in different liquid samples. It will be appreciated that the particular format chosen for performing the assay is not critical to the present invention, and that any variety of formats can be adopted with the present device.

While what has been shown and described herein constitutes a preferred embodiment of the subject invention, it should be understood that various modifications and adaptions of such embodiment can be made without departing from the present invention, the scope of which is defined in the appended claims.

CLAIMS

1. A diagnostic device for testing a liquid sample, the device comprising:

(a) a carrier for receiving at least a portion of the sample; and

(b) a sample deposition means, said sample deposition means having an injection channel that is in fluid communication with a deposition channel, said deposition channel receiving at least a portion of the sample from the injection channel, wherein the device includes a deposition channel defining surface facing the carrier and defining the deposition channel, and wherein the deposition channel has a depth that promotes lateral distribution of the sample along the distribution channel by capillary action, thereby to form a sample band on the carrier.

2. The device of claim 1, wherein the depth of the deposition channel between the deposition channel defining surface and the carrier is less than 1.0 mm.

3. The device of claim 2, wherein the depth of the deposition channel between the deposition channel defining surface and the carrier is less than 0.5 mm.

4. The device of claim 2, wherein the depth of the deposition channel between the deposition channel defining surface and the carrier is substantially 0.1 mm.

5. The device of claim 1, wherein the deposition channel is generally rectilinear.

6. The device of claim 1, wherein the deposition channel is generally curvilinear.

7. The device of claim 1, further comprising a detection channel downstream of the sample pad, wherein the sample pad has a width greater than that of the detection channel

8. The device of claim 7, including a housing enclosing the carrier with the injection channel being provided in the housing and the deposition channel defining surface being formed on the housing.

9. A diagnostic device as claimed in claim 8, wherein the housing comprises a base member and a top member, wherein the carrier is supported by the base member and held between the base and top members and wherein the deposition channel defining surface is provided on the top member.

10. A diagnostic device as claimed in claim 7, 8 or 9, wherein the carrier includes at least one detectably labeled, mobile reagent for binding with a target analyte and, at a position downstream thereof, at least one immobilized capture reagent for binding with the target analyte or a complex formed therewith.

11. A diagnostic device for testing a liquid sample, the device comprising:
(a) a carrier for receiving at least a portion of the sample; and
(b) a sample injection means, said sample injection means having an injection channel that is in fluid communication with the carrier.

12. The device of claim 11, wherein the volumetric capacity of the sample injection means provides a sufficient amount of liquid sample for the testing.

13. The device of claim 11, wherein the carrier includes at least one detectably labeled, mobile detection reagent for binding to a target analyte, and, downstream therefrom, at least one immobilized capture reagent for binding with a complex formed by a corresponding mobile detection reagent and said target analyte.

14. A diagnostic device for testing a liquid sample, the device comprising:
(a) a carrier for receiving at least a portion of the sample; and
(b) a sample delivery means, said sample delivery means

having a conduit that is in fluid communication with the carrier, said conduit having an inlet and an outlet, wherein at least a portion of the sample received at the inlet advances toward the outlet, said conduit further having a depth and a width, wherein the ratio of the width to the depth is less than 1.0.

15. The device of claim 14, wherein the width to depth ratio is less than 0.5.

16. The device of claim 14, wherein the depth of the conduit increases along the length thereof.

17. The device of claim 14, wherein the width to depth ratio at the outlet is less than the ratio at the inlet.

18. The device of claim 14, wherein the volumetric capacity of the sample delivery means provides a sufficient amount of liquid sample for the testing

19. The device of claim 14, wherein the carrier includes a sample pad, a detection pad, and a bridging pad coupling the sample pad and the bridging pad in sample flow communication.

ABSTRACT

A diagnostic device having a carrier for receiving at least a portion of a liquid sample and a sample deposition apparatus. Sample deposition apparatus has a shape corresponding to a deposition channel defining surface. Deposition channel defining surface being spaced apart from the carrier such that at least a portion of the sample disperses in a deposition channel of the sample deposition apparatus to form a sample band.

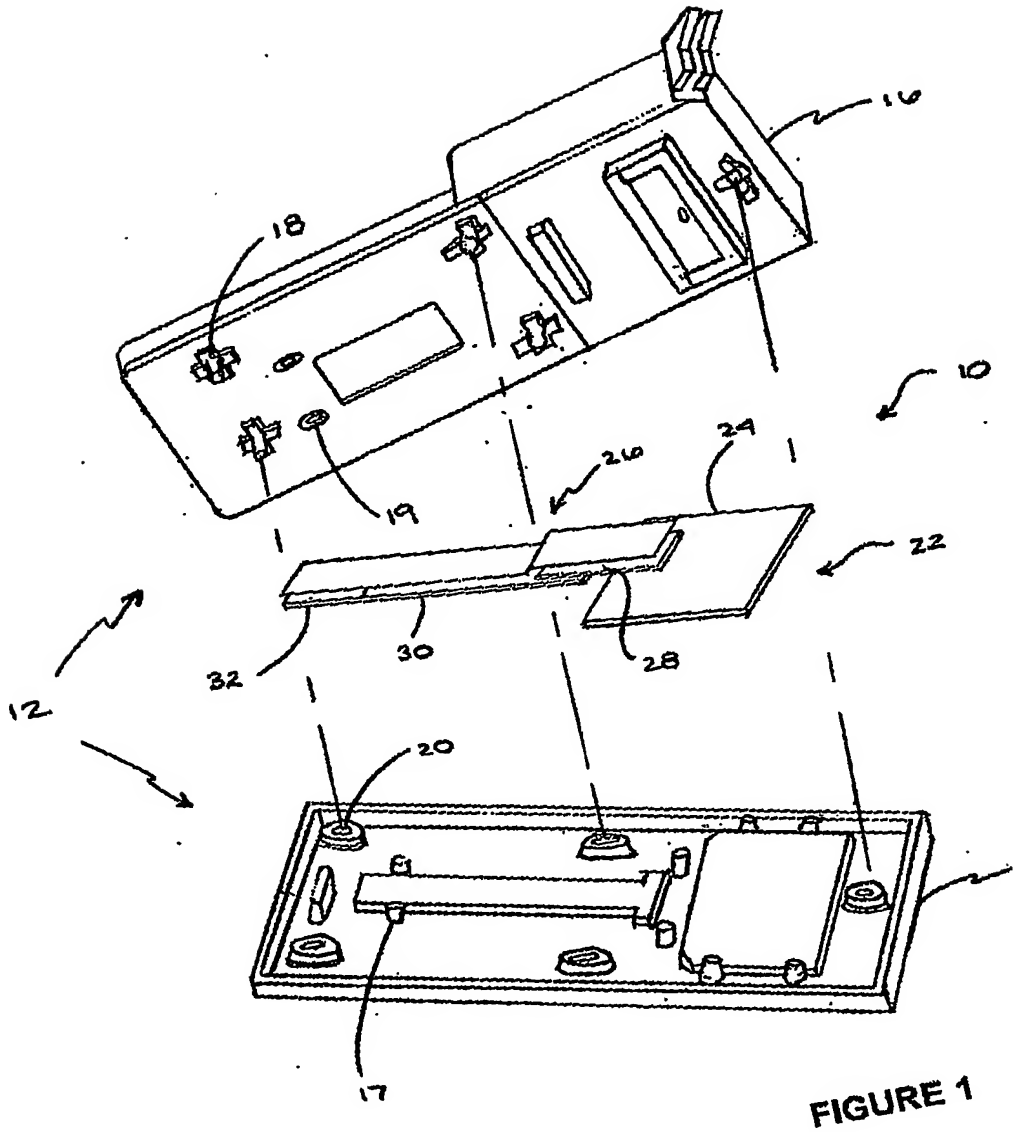


FIGURE 1

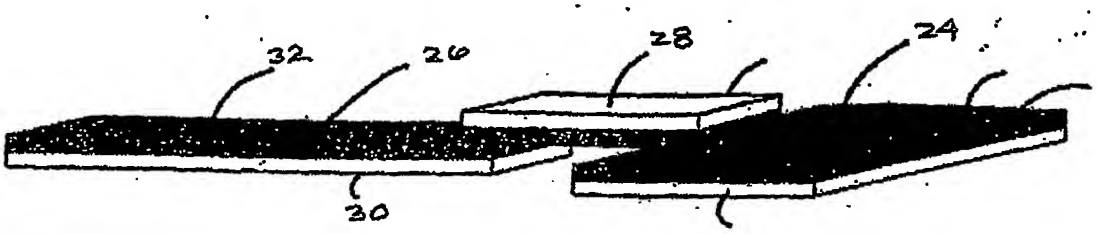


FIGURE 2

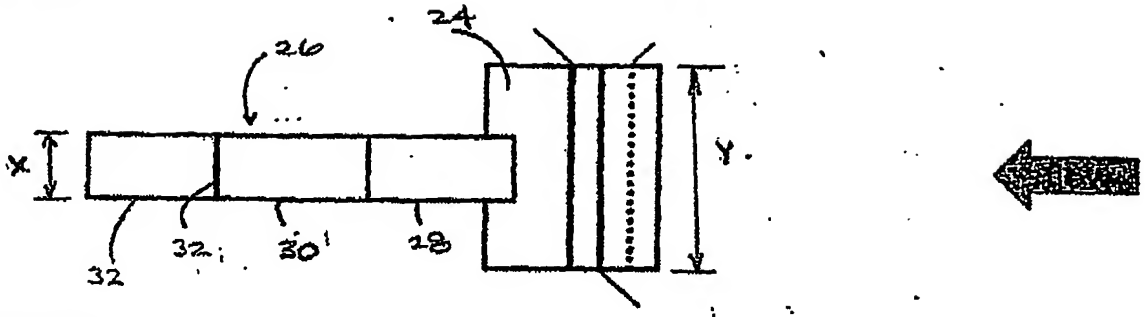


FIGURE 3

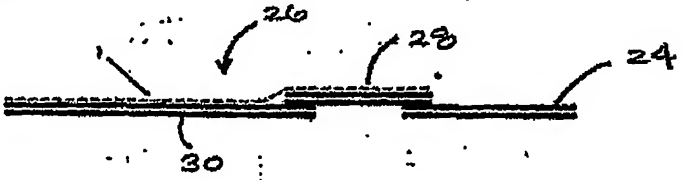


FIGURE 4

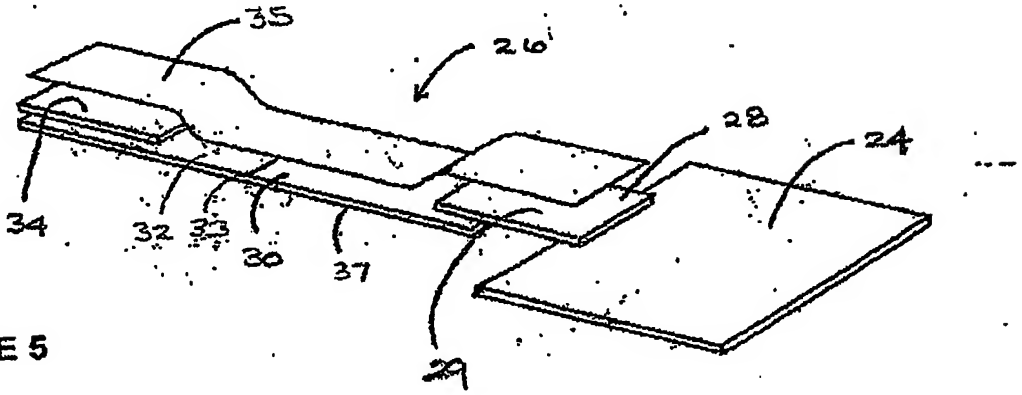


FIGURE 5

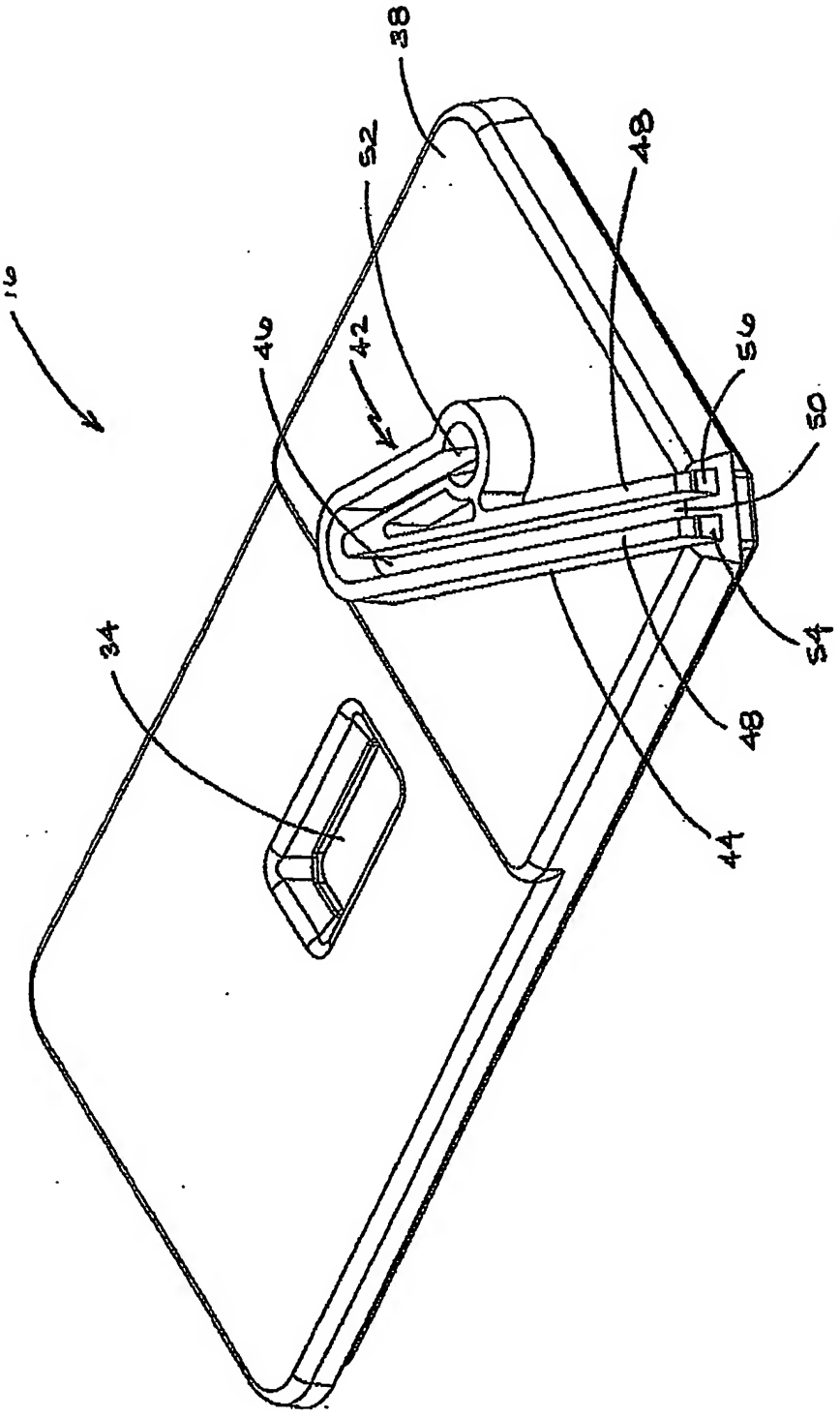


FIGURE 6

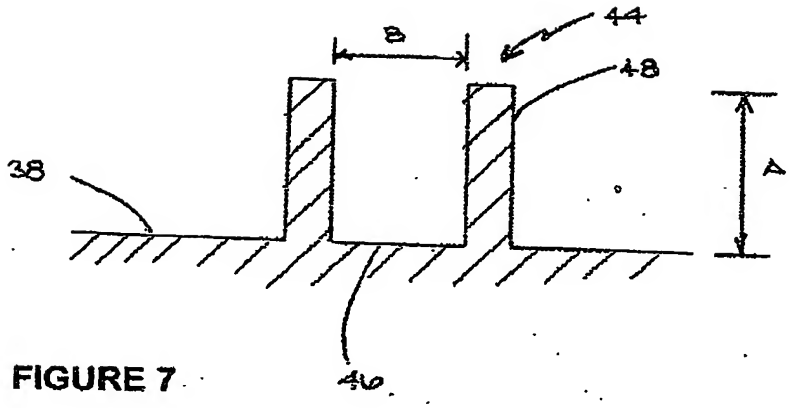


FIGURE 7

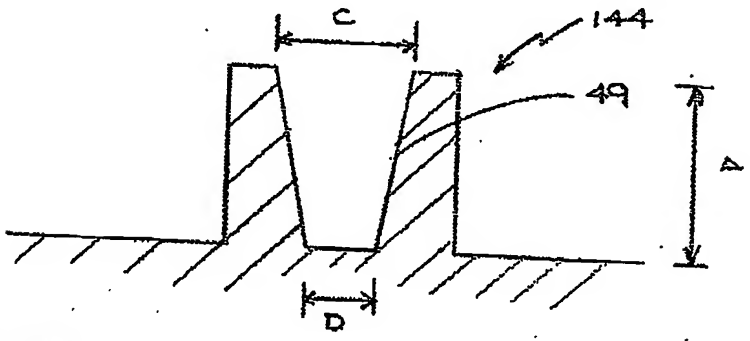


FIGURE 8

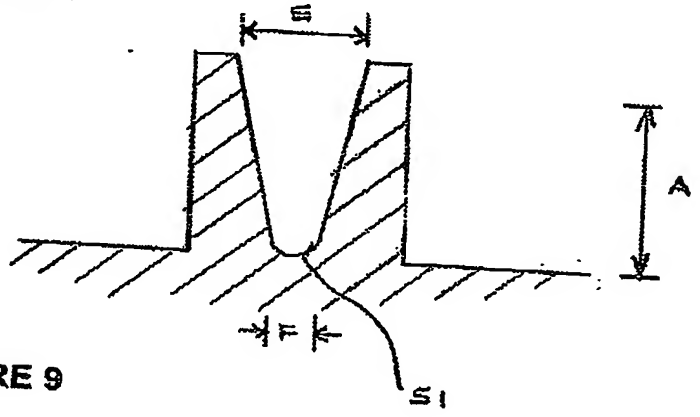


FIGURE 9

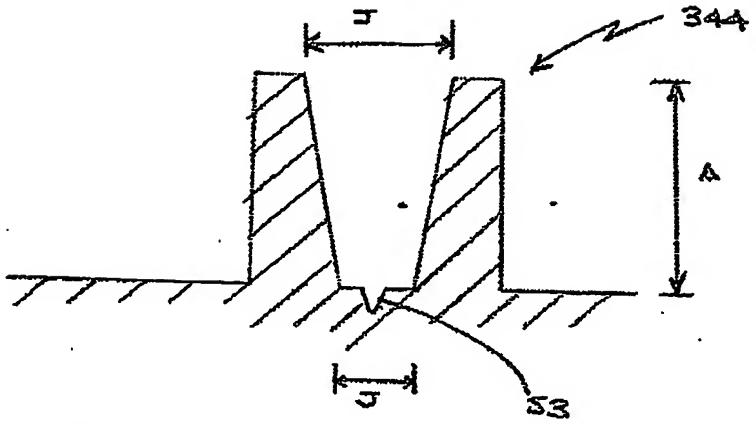
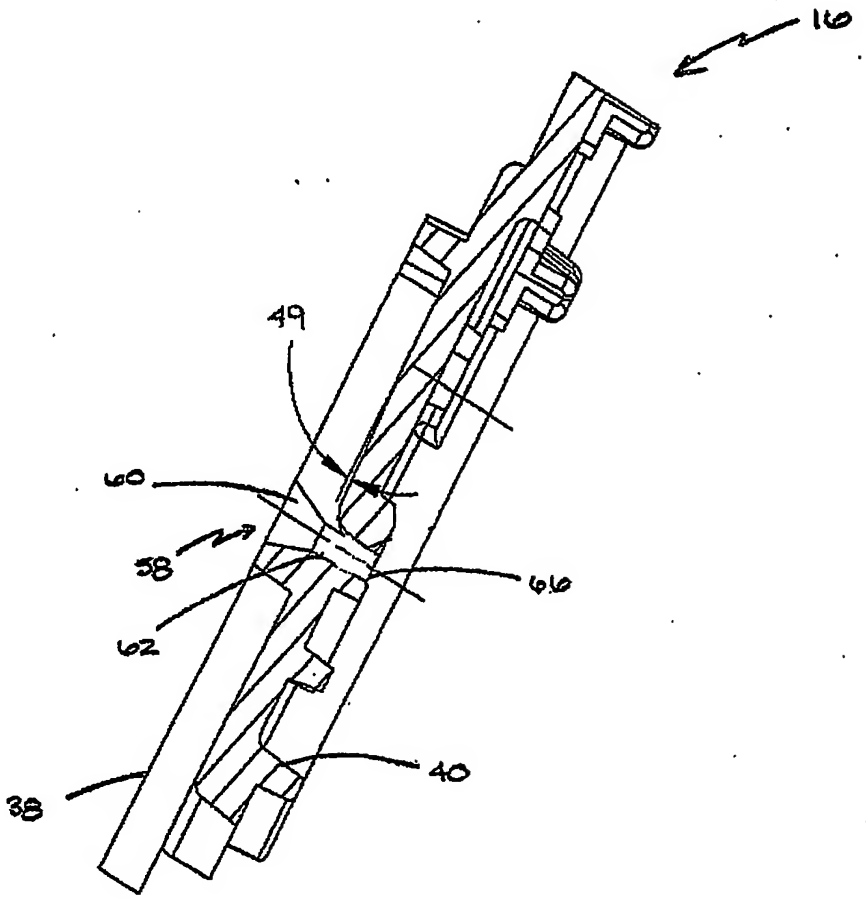


FIGURE 10



SECTION A-A

FIGURE 11

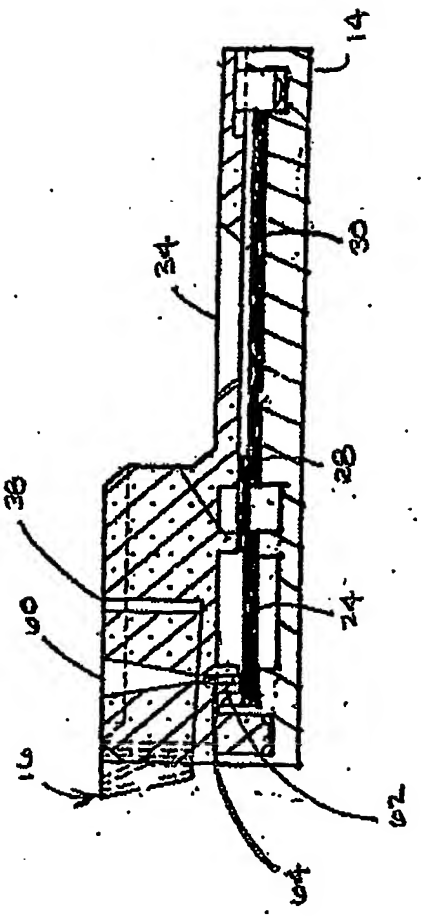


FIGURE 12

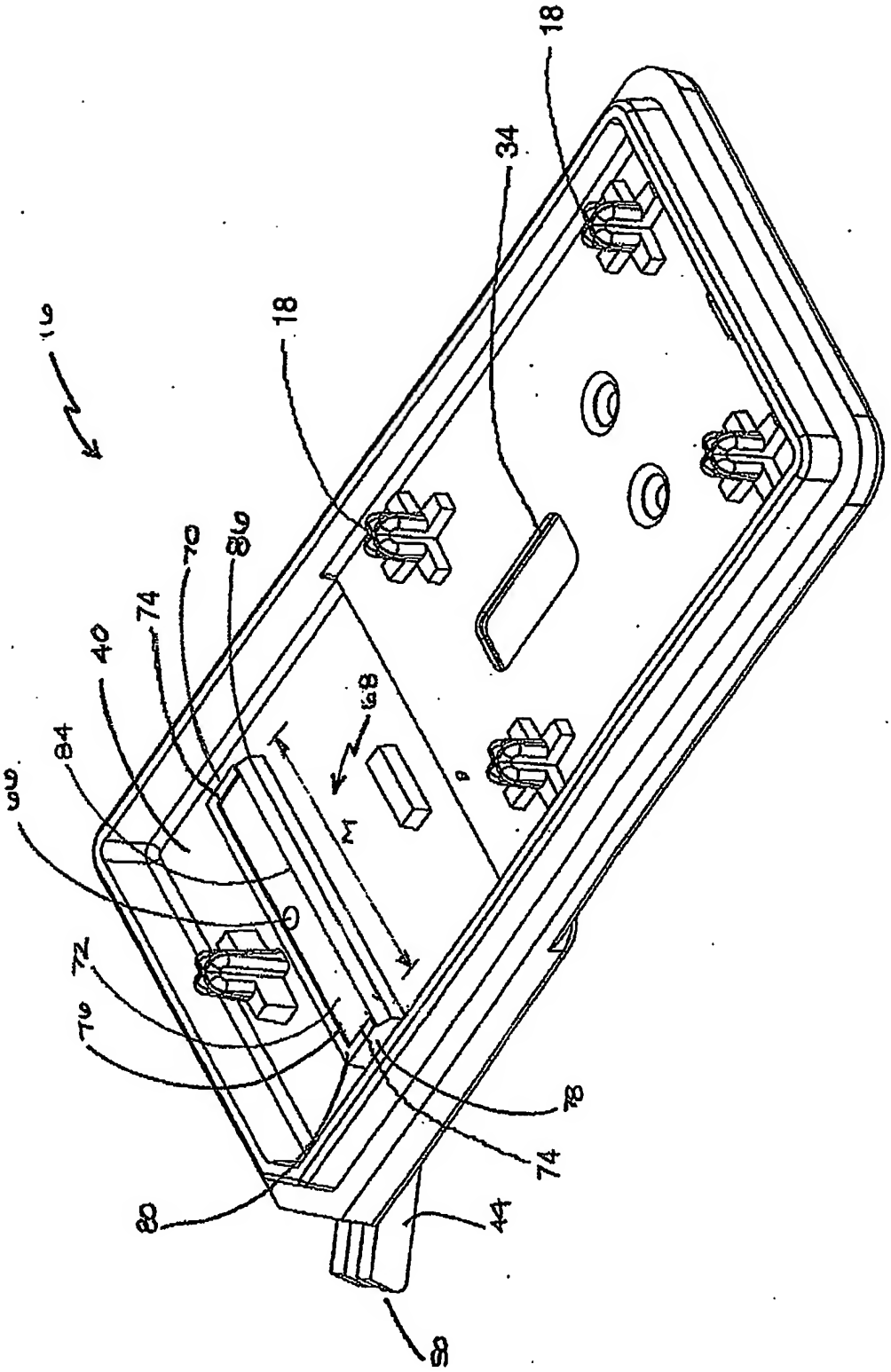


FIGURE 13

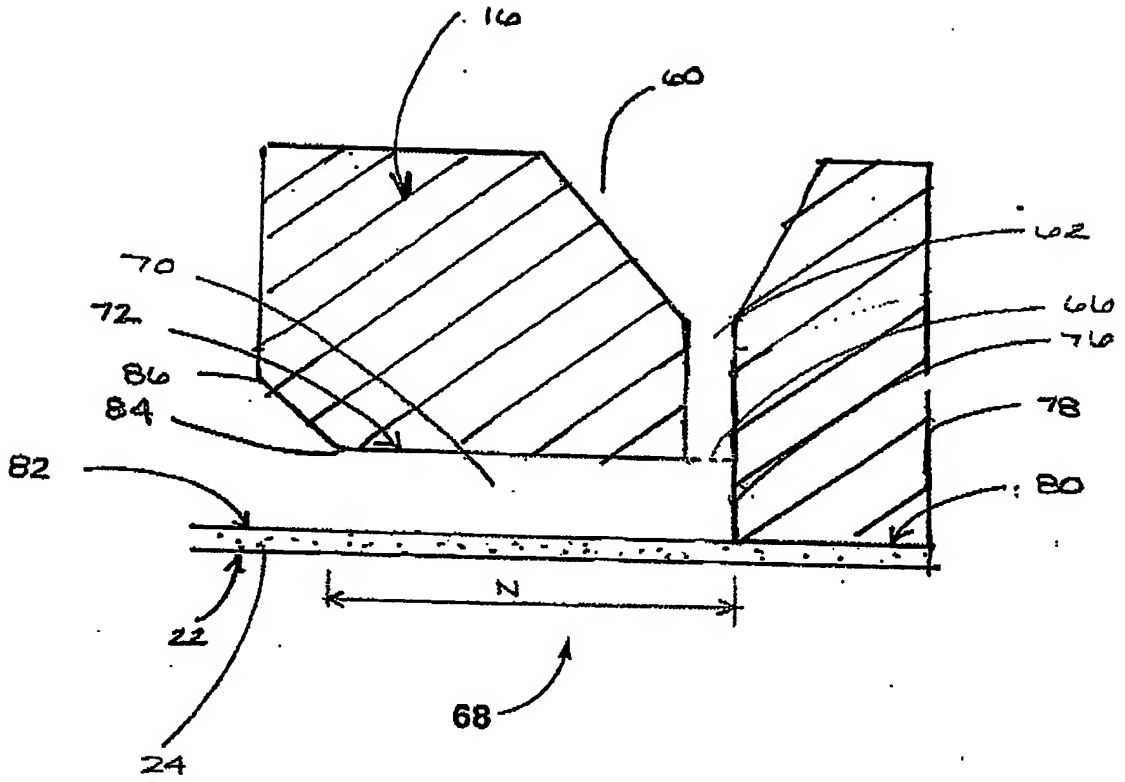


FIGURE 14

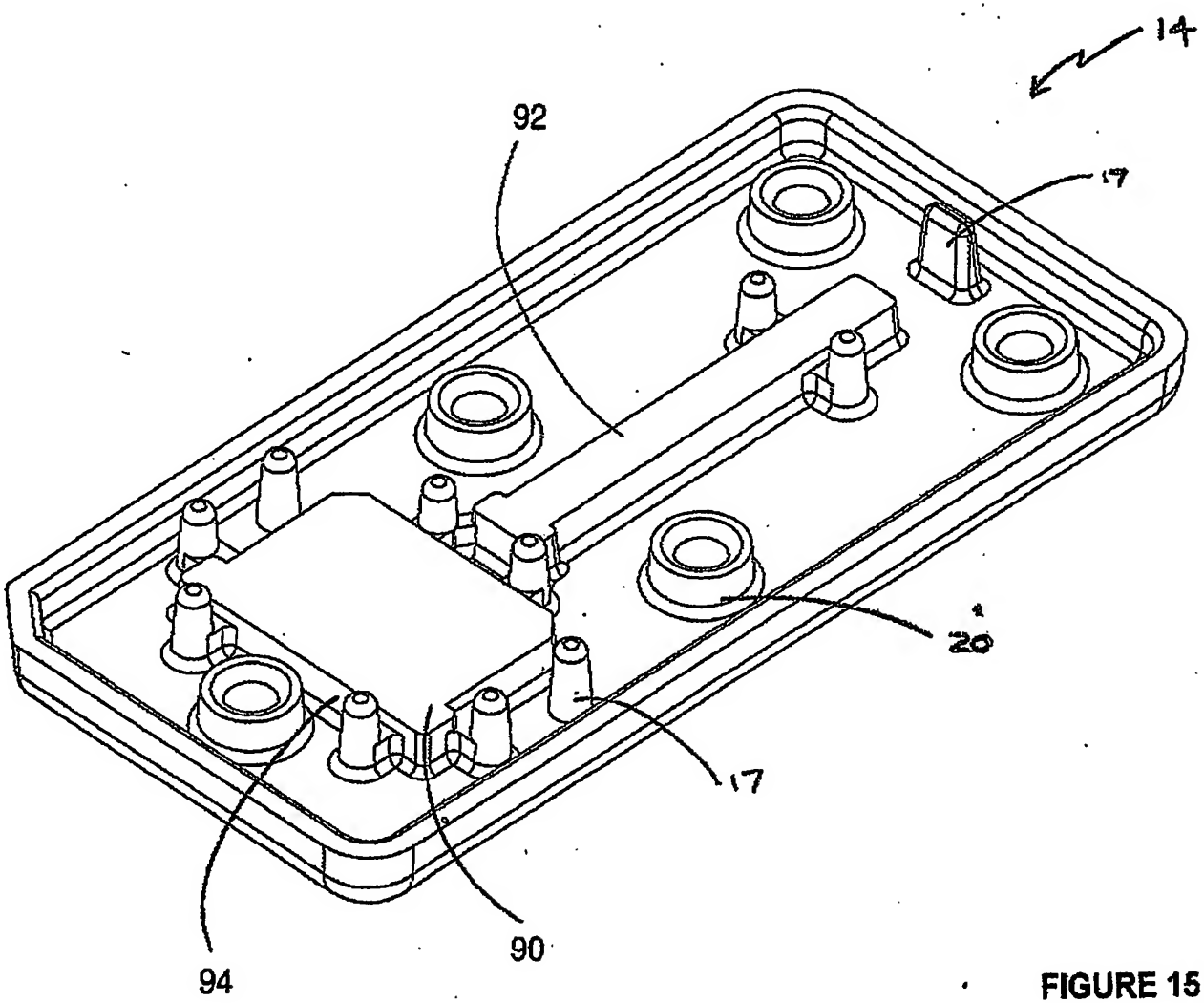


FIGURE 15

Geological Survey of Canada

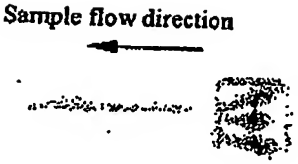


FIGURE 17

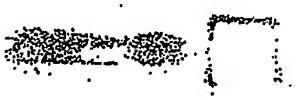


FIGURE 18

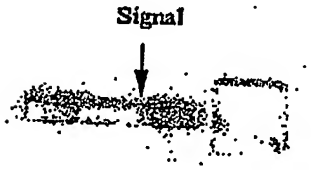


FIGURE 19

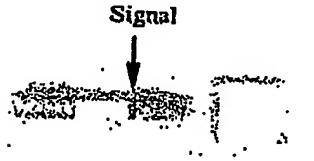


FIGURE 20

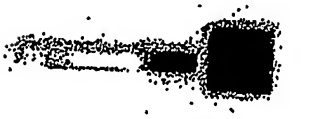


FIGURE 21